Gene Therapy for Glaucoma: Treating a Multifaceted, Chronic Disease

Teresa Borras, Curtis R. Brandt, Robert Nickells, and Robert Ritch

Glaucoma is a progressive optic neuropathy. It is characterized by a particular pattern of optic nerve head and visual field damage resulting from a number of different diseases that affect the eye. Many of these are associated with elevated intraocular pressure (IOP), which is the most common known associated risk factor, but not the only risk factor, for the development and/or progression of glaucomatous damage. The final common pathologic event is retinal ganglion cell (RGC) death.

As such, glaucoma offers a variety of potential targets for gene therapy. In the anterior segment, these include various causes of trabecular dysfunction and alterations in aqueous production. Mutations in the myocilin gene cause autosomal dominant juvenile primary open-angle glaucoma and approximately 3% of cases of adult-onset open-angle glaucomas. Several other hereditary disorders are also associated with glaucoma.

Elucidation of non-IOP-dependent risk factors for glaucomatous damage has become an area of increasingly active investigation. These risk factors are thought to be responsible for approximately 30% of open-angle glaucoma in the United States and up to 70% in Japan. They include systemic hypertension, including positional or nocturnal hypotension, cardiovascular disease, Raynaud’s disease, defective vascular autoregulation, endothelin abnormalities, sleep apnea, autoimmune disease, hemorheologic abnormalities, and cerebral microvascular ischemia. All these risk factors and their underlying causes are potentially susceptible to modulation by gene transfer.

Events leading to RGC damage and death are targets for genetic modulation. A recently described causative gene for normal-tension glaucoma, optineurin (optic neuropathy-inducing protein) is another potential target and as genetic studies continue, additional targets are likely to be identified.

Hauswirth and Beaufrere have delineated four basic prerequisites that should be met for any genetic therapy targeted to an ocular disease: an efficient and nontoxic gene delivery technique, sufficient characterization of the genetic basis of the disease to select an appropriately matched therapeutic approach, proper control of the expression of the therapeutic gene, and the availability of an animal model of the disease for preclinical testing. Glaucoma is a disease in which some of these conditions can be met. Strategies now exist that use gene therapy to modulate aqueous production and outflow and prevent RGC death.

**Target Tissues and Delivery Systems**

Delivery systems for gene therapy should be tailored to the particular disease studied. Although cell-specific targeting and prolonged gene expression are, in general, primary goals for gene transfer, situations can be envisioned in which less stringent requirements would be acceptable or even preferable. Thus, transient expression and/or neighboring approaches might be chosen over long-term and/or direct cell targeting. Each delivery system has advantages and disadvantages, and it is likely that no one vector is ideal for all strategies. Differences include tissue tropism, length of gene expression, vector carrying capacity, the potential for integration into the genome, generation of immune responses, and toxicity.

Appropriate target structures or cell types for glaucoma gene therapy include trabecular meshwork (TM), ciliary epithelium (CE), cilary muscle (CM), RGCs, and Müller cells (MCs). The TM, located at the iridocorneal angle, is a sponge-like tissue formed by different endothelial cells and extracellular matrix arranged in a particular architecture. It is the tissue responsible for maintaining the resistance to aqueous humor flow and keeping the ocular globe inflated. The CE is formed of two, intimately connected cell layers covering the ciliary processes. The outer layer facing the posterior chamber is nonpigmented, and it is responsible for the secretion of aqueous humor. Once secreted, the aqueous humor flows around the iris to the anterior chamber and leaves the eye through the cells of the TM and inner wall of Schlemm’s canal to the venous system. The CM lies close to the CE and the TM. When the CM contracts, it provides lens accommodation and changes in the configuration of the TM, facilitating the outflow of aqueous humor. The RGCs, the innermost cells of the retina, are responsible for sending the transduced light signal to the brain through their axons, which make up the optic nerve (ON). Damage and death of the RGCs with subsequent loss of ON fibers is triggered by a number of insults, most commonly, elevated IOP.

To date, six delivery systems have been tested for ability to deliver genes to the relevant tissues or cells. These are summarized in Table 1 and include adenoviruses (Ads), adenoassociated viruses (AAVs), herpes simplex viruses (HSV), lentiviruses (LVs; feline immunodeficiency virus [FIV]) and human...
immunodeficiency virus (HIV), liposomes (LPs), and naked DNA.

**Anterior Segment**

Several studies have established that Ad vectors can deliver transgenes very efficiently to the TM after intracameral injection. Because of the natural flow of aqueous humor, intracameral delivery of vectors carries the viruses directly to the TM. Recombinant Ads transduce all TM cell types with high efficiency in all species so far investigated (rabbits, mice, rats, dogs, monkeys, and anterior segments from postmortem human donors). Report gene expression driven from the cytomegalovirus (CMV) major immediate early gene promoter has been reported to last from 1 week to at least 1 month.

Delivery to other cell types, such as iris and corneal endothelial cells, also occurs, but with reduced efficiency. In addition, delivery of Ad vectors expressing green fluorescent protein (GFP) permits noninvasive monitoring of delivery and length of gene expression, which is particularly important in primate studies in which it could reduce the need for killing the animal to obtain data. Multiple deliveries to the anterior chamber of an Ad vector expressing GFP have been achieved with doses that do not induce infiltration of immune cells into the anterior chamber. There are 47 known human Ads with different sets of antigens (serotypes). Use of vectors derived from different serotypes may result in altered tissue tropism and delivery to different cell types. In addition, modifying the Ad fiber protein to alter cell specificity may be possible.

**AAV vectors** appear to be unsuitable for anterior segment delivery, because transduction has not been demonstrated to date with vectors derived from serotypes 2, 3, and 4 (Borrás, Samulski, Kaufman, unpublished data, 2001). Whether other serotypes are capable of transducing TM and CE remains to be determined. It may also be possible to modify the AAV attachment protein to target specific cell types.

HSV vectors are capable of efficient gene delivery to structures and cells relevant to glaucoma. In monkeys and rodents, intracameral injection results in efficient delivery to cells in the TM and CE. Other cell types, such as iris pigmented epithelial and corneal endothelial cells, are also transduced, but at reduced efficiency. Only the CMV promoter has been tested with HSV vectors, and gene expression has lasted only 10 days, at most. It will be interesting to determine the mechanism involved in this phenomenon. Vector DNA persists at least 1 month after transduction, suggesting that a promoter shutoff is involved.

Delivery with LV vectors based on HIV and FIV has also been tested. These vectors must be pseudotyped by replacing the envelope gp120 with the vesicular stomatitis virus envelope glycoproteins to broaden the host range. Efficient delivery occurs in the TM with both types of vectors. One potential advantage of LV vectors is the ability to integrate into the host cell genome of nondividing or slowly dividing TM cells, which may result in increased duration of expression. However, HSV episomal elements are also stable in nondividing cells, particularly neuronal cells; thus, integration may not be needed for some uses. The major drawback to integrating vectors is the potential for insertional mutagenesis, which although rare, is a serious concern.

Liposome-mediated plasmid delivery has also been achieved. TM cells are transduced, but the efficiency is low; thus, this delivery method must be improved. It should be noted that there are circumstances in which low delivery efficiency or low-level expression of the transgene may be acceptable or even desirable, so that low-efficiency vectors should not be excluded from consideration. Toxicity due to certain types of liposomes or specific constituents has also been seen and alternative preparations must be identified.

Transfer of naked DNA to anterior chamber structures has not been reported to date, although corneal delivery by this

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**Table 1. Glaucoma Relevant Tissues and Available Vector Systems**

<table>
<thead>
<tr>
<th>Tissue or Cell Type</th>
<th>Vector</th>
<th>Route</th>
<th>Species</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular meshwork</td>
<td>Adenovirus</td>
<td>Intracameral</td>
<td>Rabbit, rats, mouse, dog</td>
<td>High</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Intracameral</td>
<td>Monkey, oc-human</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>serotypes 2, 3, 4</td>
<td>Tissue culture</td>
<td>Human</td>
<td>No transduction</td>
<td></td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Intracameral</td>
<td>Rodent, monkey,</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Intracameral</td>
<td>Oc-human</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Liposomes</td>
<td>Intracameral</td>
<td>Rat, monkey</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Ciliary epithelium</td>
<td>Adenovirus</td>
<td>Intracameral</td>
<td>Oc-human</td>
<td>High</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Lens culture</td>
<td>Rat</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Intracameral</td>
<td>Rodent, monkey,</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Tissue culture</td>
<td>Human</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Liposomes</td>
<td>Tissue culture</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Ciliary muscle</td>
<td>Adenovirus</td>
<td>Tissue culture</td>
<td>Human</td>
<td>Good</td>
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<tr>
<td>Adeno-associated virus</td>
<td>Tissue culture</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Tissue culture</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Tissue culture</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Liposomes</td>
<td>Tissue culture</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Retinal ganglion cells</td>
<td>Adenovirus</td>
<td>Intravitreal</td>
<td>Rodent</td>
<td>Poor</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Intravitreal</td>
<td>Rat</td>
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<td>Herpes simplex virus</td>
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<td>Liposomes</td>
<td>Tissue culture</td>
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</tr>
</tbody>
</table>

UP, unpublished observations.
method has been used. Successful delivery of naked DNA to cells at the time of glaucoma filtration bleb surgery has also been achieved; this potential target for therapy is discussed later in this report.

Efficient gene delivery to the CM in vivo has not been reported to date. It is not clear whether this is due to investigators’ failure to consider this tissue or whether the CM is refractory to transduction. The latter is more likely, because whole eye sections are usually scanned in delivery studies, and CM delivery would be noticeable with either β-galactosidase or GFP staining. Nevertheless, CM cells can be transduced in culture, at least with HSV vectors. Transduction of muscle cells has been shown to be inhibited by the presence of the sheath surrounding muscle fiber bundles.

**Posterior Segment**

It is now well established that intravitreal delivery is the preferred route to deliver genes to the RGCs. Intravitreal injection of Ad vectors results in efficient delivery to MCs. For reasons not yet clear, Ad gene transfer to the RGCs is very limited. MCs however, appear to be important sources of neurotrophic factors, and delivery of such secreted factors or modulation of their expression in these cells could be important in neuroprotective strategies to protect RGCs (discussed later).

AAV appears to have selective, stereotype-specific tropism for the RGCs. Intravitreal injections in the rat result in a 72% transduction efficiency, with optimal expression occurring between 2 and 4 weeks after injection in rodents. This high efficiency may be due to the expression of membrane-associated heparin sulfate proteoglycans in RGCs, which mediate attachment of AAV to cells. Heparin sulfate proteoglycans are also receptors for HSV.

Delivery of HSV results in efficient transduction of RGCs. Efficiencies of up to 50% can be achieved with a single intravitreal injection in rats and multiple injections, even at high doses, have also been reported. Delivery to RGCs with HSV vectors has also been achieved in primates, but transduction is localized to the delivery site (cannula placed close to the RGC layer). Transduction of rat RGCs by HSV after injection into the superior colliculus of rats have been negative (Nickells and Brandt, unpublished data, 2000, 2001). However, this reduces transduction efficiency, particularly with HSV vectors. Both AAV and LV vectors do not appear to induce inflammation, but most of these studies have been performed in rodents, and it is clear that species differences also play a role. HSV vectors, even at high doses, do not induce inflammation in rats. In contrast, delivery of equivalent amounts of HSV vector to the anterior chamber of primates induces a significant, but transient, response. Although the response clears without any apparent long-term effects, reducing or eliminating the response would be beneficial.

**Potential Target Genes for Relevant Tissues**

Although the genetic basis of most glaucomas remains unknown, the transfer and expression of genes encoding IOP-lowering and/or neuroprotective gene products may serve to modify the physiology of relevant cells and block the pathogenesis of the disease. As with other chronic diseases, the use of genes to treat glaucoma will provide improved efficiency and a longer duration of effect.

Lowering IOP by manipulating the tissues of the anterior segment with gene therapy could represent the first immediate treatment of glaucoma. The currently available drugs must be administered daily, and compliance is a major problem. Gene therapy could alleviate this problem. The TM, CE, and CM are all potential targets. The TM’s juxtacanicular region and inner wall of Schlemm’s canal constitute the primary barrier to aqueous humor before it leaves the eye. Manipulation of the biochemistry of the cells and extracellular matrix in these regions has the potential to modulate outflow resistance and lower IOP. Investigators have successfully transferred genes to this tissue with different vectors and through different routes of administration (Table 1). Several investigators have begun to deliver potential physiologically relevant genes, in addition to reporter genes (Table 2). Kee et al. have demonstrated that an Ad vector carrying the metalloproteinase stromelysin can be transduced to TM cells of rats after intracameral injection. In human postmortem perfused organ cultures, Ad vectors carrying wild-type myoctin and genes that affect the cytoskeleton increase outflow facility compared with vehicle-injected control cultures. Efforts to study reduction of elevated IOP by modulating TM resistance have been hampered by the lack of an animal model that does not involve scarring this tissue. Monitoring reduction of IOP in normotensive animals is more difficult than assessing IOP changes in hypertensive ones. Gene transfer to the TM offers the unique possibility of creating such a model by transducing genes that increase rather than reduce IOP.

Aqueous humor, which contains numerous factors that can signal the cells of the TM and modulate their resistance characteristics, is produced and secreted by the CE. Efforts to identify relevant genes have revealed that the CE has neuroendocrine activity, releasing hormones and regulatory peptides. There may also be a light sensor in the CE that plays a role in the diurnal variation of IOP. Manipulation of the concentration of such CE factors may be an attractive target for glaucoma gene therapy.
neurotrophic factor (BDNF) has been shown to protect RGCs from apoptosis. Ad-mediated intravitreal delivery of brain-derived neurotrophic factors (bDNF) has been shown to protect RGCs in a rat optic nerve transection model.40 MCs are transduced by Ad and enhancement of RGC survival is due to secretion of bDNF from these cells. Even though the expression of the transgene was prolonged to 1 month by the use of immuno-suppressants, the protective effect was transient. A recent study has demonstrated that the bDNF receptor TrkB is markedly downregulated after axotomy of the optic nerve.41 Furthermore, AAV-mediated TrkB gene transfer into RGCs combined with exogenous bDNF administration markedly increases neuronal survival. Seventy-six percent of RGCs remained alive at 2 weeks after axotomy, a time when more than 90% of these neurons are lost without treatment in damaged RGCs.41 Isenmann et al.54 also found protection of the RGFs after Ad-mediated delivery of bDNF, and protection was increased by the combined systemic administration of the free radical scavenger tert-butyl-(2-sulfophenyl)-nitrone (S-PBN). Similar RGC survival results were obtained recently with Ads containing the ciliary neurotrophic factor (CNTF).55 Because some of the changes associated with optic nerve axotomy are not the same as those observed in these glaucoma, these exciting results must be observed by experiments in which the RGC insult is more physiologically related to glaucoma (elevated IOP).

Gene therapy could also be used to prevent the proliferative wound-healing response that follows glaucoma filtration surgery. Using an Ad vector encoding the cell cycle inhibitor p21 (rAd-p21) Perkins et al.56 inhibited proliferation of Tenon’s fibroblasts in rabbits with a single administration of rAd-p21 (by applying a vector-soaked sponge for 5 minutes). This resulted in the maintenance of functional filtration blebs at 30 days after surgery without the severe tissue effects observed in the mitomycin-treated eyes. Other investigators have used naked DNA to transfer the reporter gene chloramphenicol acetyl-transferase to the same cells.57

The final common outcome of glaucoma is RGC death. The stimulatory mechanism or mechanisms causing RGC death are unclear, but several strategies to block RGC apoptosis are available. Repeated intraocular injections of neurotrophic factors temporarily rescue RGCs from axotomy-induced death.50–53 To overcome the problems associated with frequent repeated injections of these proteins, several investigators are now exploring the use of gene transfer to deliver such products. Ad-mediated intravitreal delivery of brain-derived neurotrophic factor (bDNF) has been shown to protect RGCs in a rat optic nerve transection model.40 MCs are transduced by Ad and enhancement of RGC survival is due to secretion of bDNF from these cells. Even though the expression of the transgene was prolonged to 1 month by the use of immuno-suppressants, the protective effect was transient. A recent study has demonstrated that the bDNF receptor TrkB is markedly downregulated after axotomy of the optic nerve.41 Furthermore, AAV-mediated TrkB gene transfer into RGCs combined with exogenous bDNF administration markedly increases neuronal survival. Seventy-six percent of RGCs remained alive at 2 weeks after axotomy, a time when more than 90% of these neurons are lost without treatment in damaged RGCs.41 Isenmann et al.54 also found protection of the RGFs after Ad-mediated delivery of bDNF, and protection was increased by the combined systemic administration of the free radical scavenger tert-butyl-(2-sulfophenyl)-nitrone (S-PBN). Similar RGC survival results were obtained recently with Ads containing the ciliary neurotrophic factor (CNTF).55 Because some of the changes associated with optic nerve axotomy are not the same as those observed in these glaucoma, these exciting results must be observed by experiments in which the RGC insult is more physiologically related to glaucoma (elevated IOP).

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**FUTURE DIRECTIONS**

In summary, it is clear that several systems are available for gene delivery to tissues relevant for glaucoma gene therapy. It is also clear that multiple therapeutic strategies are available to affect aqueous production and outflow to modulate IOP and to protect RGCs from apoptosis. Thus, the field of glaucoma gene therapy is poised to provide significant advances in alleviating blindness due to this disease. However, additional work is needed to improve gene delivery for glaucoma. We need a better understanding of the role of the extracellular matrix, cytoskeletal remodeling, and cell-signaling events in the TM. An improved understanding of the regulation and production of aqueous humor, and the factors released from the CE that could modulate TM function is needed. We need an improved understanding of the cell death pathways activated in RGCs after various stimuli and under conditions of elevated and nonelevated IOP. Animal models that mimic alterations in the TM and CE leading to elevated IOP are sorely needed to test therapies. We also need a better understanding of the innate and antigen-specific immune responses to the various vector systems. Understanding the differences in such responses between rodents and primates is essential in reducing these types of responses. Efforts to identify additional genes involved in glaucoma will provide important clues for additional gene therapy strategies. Finally, it is critical to understand why ectopic gene expression from the various vectors shuts off in various ocular cells and to identify promoters or other gene expression elements that will provide long-term expression of the transgenes.

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